Does stronger pollen competition improve offspring fitness when pollen load does not vary?

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Abstract

Premise of the study: Competition among pollen grains from a single donor is expected to increase the quality of the offspring produced because of the recessive deleterious alleles expressed during pollen-tube growth. Evidence for such an effect is inconclusive, however. Furthermore, a large number of studies suffer from confounding variation in pollen competition with variation in pollen load.

Methods: In this study, we tested the effect of pollen competition on offspring performance independently of pollen-load variation. We compared seed mass and early seedling performances in *Dalechampia scandens* (Euphorbiaceae) between crosses in which variation in pollen competition was achieved, without variation in pollen load, by manipulating pollen grains dispersion on the stigmas.

Key results: Despite a large sample size (211 crosses on 20 maternal plants), we failed to find an effect of pollen competition on seed characteristics or early seedling performance. Paternal effects were always limited and pollen competition never reduced the within-father (residual) variance.

Conclusion These results suggest that limited within-donor variation in genetic quality of pollen grains reduces the potential benefits of pollen competition in the study population. The lack of paternal effects on early sporophyte performance further suggests that benefits of pollen competition among pollen from multiple donors should be limited as well, and it raises questions about the significance of pollen competition as a mechanism of sexual selection.

Key words: evolvability, germination, paternal effect, pollen tube growth, prezygotic selection, sexual selection

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Introduction

Pollen competition occurs whenever the number of viable pollen grains deposited on the stigma is larger than the number of available ovules (Haldane, 1932). Because a large number of the microgametophytic genes are expressed during pollen-tube growth, competition among haploid pollen is expected to be an important source of selection against deleterious recessive mutations (Mulcahy, 1979; Walsh and Charlesworth, 1992). Consequently, plants may benefit from pollen competition by producing offspring of higher quality. The importance of pollen competition is supported by the various floral features that potentially increase it, such as elongated pistil (Mulchahy, 1979; Lankinen and Skogsmyr, 2001), functional syncarpy (Armbruster et al., 2002), enlarged stigmatic surfaces (Armbruster, 1996), and delayed stigma receptivity (Galen et al., 1986; Lankinen et al., 2007; Lankinen and Madjidian, 2011; see Skogsmyr and Lankinen, 2002 for a review). Evidence for beneficial effects of pollen competition have been provided by studies demonstrating better performance of offspring produced under intense competition among pollen from either single (Mulcahy and Mulcahy 1975; Palmer and Zimmerman 1994; Queseda et al. 1993) or multiple donors (Mulcahy and Mulcahy 1987; Winsor et al., 1987; Quesada et al., 1996; see Delph and Havens, 1998 for review; but see Snow, 1990). Pollen competition has also been suggested to reduce the negative effects of inbreeding by increasing the fitness of progeny obtained by self-fertilization (Armbruster and Rogers, 2004; Lankinen and Armbruster, 2007).

If pollen competition increases the quality of the offspring produced, it may also reduce the within-father variation in offspring quality (Schlichting et al., 1987; Hormaza and Herrero, 1992), and consequently increase our ability to detect paternal effects, that is, systematic phenotypic differences among offspring from genetically different fathers (Mazer and Gorchov, 1996). Paternal effects are usually estimated as the proportion of the offspring phenotypic variance explained by the identity of the father. These effects on early

sporophytic performances are often small and difficult to detect (Mazer and Gorchov, 1996). A decrease in the within-father variation via pollen competition could therefore help uncovering paternal effects by decreasing the uncertainty in the paternal variance. In this study, we therefore test whether competition among pollen from single donor affects the average and the within-father (residual) variation of early life history performance traits of offspring.

We tested the effects of pollen competition on the mean and the variance of early sporophyte performance in the Neotropical vines *Dalechampia scandens* (Euphorbiaceae). In most *Dalechampia* species, female flowers bear an elongated style with an enlarged stigmatic surface that extends part way down the style (Armbruster et al., 1995; Armbruster, 1996). Pollen grains that land on the lateral stigmatic surface need to grow their pollen tube first to the stylar tip (i.e. away from the ovaries) where they bend 180º to finally grow back in the direction of the ovaries (Armbruster et al., 1995). We took advantage of this particular feature of the female flower in *D. scandens* to manipulate the level of pollen competition while keeping the pollen load constant by varying the dispersion of the pollen grains on the stigma. Indeed, early studies of pollen competition have been criticized for confounding variation in pollen competition with variation in pollen load (Mulcahy and Mulcahy, 1975; Charlesworth, 1988; Delph and Havens, 1998; Paschke et al., 2002; Bernasconi et al., 2003), and it has been repeatedly advocated (but rarely achieved; see discussion) that the effects of pollen competition on offspring vigor should be studied at constant pollen load (e.g. Lassere et al., 1996). In our study, strong pollen competition was achieved by low dispersion of the pollen grains deposited on the tip of the stigma and weak competition was achieved by high dispersion of the pollen grains along the lateral stigmatic surface.

Methods

Study species

Dalechampia scandens (Euphorbiaceae) is a self-compatible Neotropical vine bearing pseudanthial inflorescences (blossoms), which are pollinated by female apid and/or megachilid bees (Armbruster, 1985). The *Dalechampia* blossom comprises one male and one female subinflorescence, together subtended by showy involucral bracts. The male subinflorescence comprises ten staminate flowers and a gland producing terpenoid resin collected by visiting bees that use resin in nest construction (Armbruster, 1985). The female subinflorescence comprises three female flowers containing three ovules each and produces a maximum of nine seeds. When blossoms are oriented naturally, female flowers are situated below the male flowers, and may receive autogamous pollen on their stigma when male flowers open, although different geometries of the blossom may ensure some degree of herkogamy (Armbruster et al., 2009).

The greenhouse population on which this experiment was conducted was started from seeds collected in 1998 from 75 maternal plants from a single population in Quintana Roo, Mexico (20 °13'N, 87°26'W). A population of minimum 200 individuals was generated and maintained by outcrossing while keeping track of the pedigree of all individuals at each generation. The individuals used in this study belong to the fifth greenhouse generation.

Experiment

Manual crosses were performed between 10 (designated) male and 20 (designated) female plants arranged in two blocks of five males and 10 females each. Each "female plant" was crossed with each of the five "male plants" under strong or weak pollen competition. In total we performed more than 200 crosses (10 males crossed with 10 females each under 2 pollination treatments), the crosses that failed to produce seeds being replicated. Crosses were

performed between individuals with a mean coefficient of relatedness of 0.01 (range 0 - 0.02) based on their pedigree. Blossoms with receptive female flowers but closed male flowers (female phase) were emasculated by removing the whole male sub-inflorescence. Each of the three female flowers was pollinated by brushing a freshly opened staminate flower from one of the five male plants on the stigma of each of the three female flowers. Difference in pollen-competition intensity without difference in pollen load was obtained by either spreading the pollen evenly on the lateral surface of the stigma (high pollen dispersion, Fig. 1A), or by placing the pollen on the tip of the stigma (low pollen dispersion, Fig. 1B). Under high pollen dispersion, pollen competition is reduced because the probability of a pollen grain to fertilize the ovule strongly depends on its position on the surface of the stigma. Under low dispersion, strong pollen competition is expected because the fertilization success of each pollen grain mainly depends on the growth rate of the pollen tube. To ensure that the amount of pollen deposited on the stigma was sufficient and similar in the both treatments, we counted roughly the number of pollen grains deposited on each stigma using a hand-lens (median = 180 range ca. 150 to 250).

Pollinated blossoms were bagged in order to collect the seeds after explosive dehiscence five to six weeks later (Armbruster, 1982). For each cross, we recorded the time it took for the seeds to mature (*seed maturation time*), that is, the number of days from pollination to dehiscence. After dehiscence, seeds (n = 1726 from 211 blossoms) were collected and individually weighted on a precision balance (to the nearest 0.001g) 2 to 3 days after harvest. *Blossom seed set*, the number of seeds produced by the blossom, was also recorded. Size variation among blossoms on the same maternal plant may affect the mass of the seeds produced independent of the pollination treatment. We therefore measured the *diameter of the blossom peduncle* as a proxy of the blossom size and used it as covariate in the statistical analysis.

148 Seeds were stored in a dry environment and, in the fall of 2014, they were sown in
149 germination trays with individual compartments. Each tray contained 54 separated
150 compartments filled with soil (sphagnum mixture) with high humidity level. Seeds from the
151 different crosses where placed randomly (one seed per compartment) among trays and the
152 trays (32 in total) were placed randomly on two tables in one room in the greenhouse, and
153 covered with a plastic sheet to maintain humidity. We also moved the trays on each table to
154 diminish positional effects. One month after sowing the seeds, we recorded the *germination*
155 *success* and several variables related to seedling vigor; the *diameter of both cotyledons*, the
156 *number of true leaves* longer than 10 mm, and the *length of the largest leaf*. In a previous
157 study, we showed that the length of the largest leaf was strongly correlated with the biomass
158 of the seedling (Pélabon et al., 2005). Because cotyledon size is correlated with the reserves
159 available in the seed (Stanton, 1984), while the size of the largest leaf reflects the early
160 growth of the seedling, we estimated paternal effects on these two traits separately.
161
162 *Statistics*
163 For each response variable, we first tested the effect of the treatment by comparing mixed-
164 effect models that included both father and mother identity as random factors. Blossom
165 identity nested in mother was also included as a random factor in the analysis of seed mass.
166 For the analysis of seed maturation time we included blossom seed set as a covariate. For the
167 analysis of the seed mass, peduncle diameter, blossom seed set and maturation time as well as
168 their interaction with treatment were included as covariates. We compared models using
169 Akaike Information Criterion (AIC) values obtained from models fitted by maximum
170 likelihood (ML). Estimates for the fixed effects were subsequently obtained from the highest
171 ranked models fitted using restricted maximum likelihood (REML). We then tested the
172 statistical significance of the paternal effects by comparing the highest ranked models with

similar models that did not include paternal identity as a random factor. Comparison of models with different random structures was done on models fitted by REML (Zuur et al., 2009).

To estimate the paternal and maternal effects (independently of their statistical significance) and the residual variance, we ran variance components analyses for the two treatments separately. Variance component analyses were performed by fitting mixed-effect models with the R package MCMCglmm (Hadfield, 2010) in order to obtain credible intervals for the estimates of the random variances. As priors for the Bayesian mixed models, we used zero-mean gaussian distributions with very large variances (10^8) for the fixed effects, scaled F-distributions where the variance/1000 were $F_{1,1}$ distributed for the variance parameters, and inverse-Wishart distributed for the residuals (Hadfield, 2010). These models ran for 260,000 iterations, with a burn-in phase of 10000 and a thinning interval of 250 iterations for a total of 1000 samples of the posterior distribution. Maternal and paternal effects were estimated only for maturation time, seed mass, cotyledon diameter and the size of the largest leaf at one month, and not for blossom seed set because it had very little variation. We found no evidence for an effect of the pollen competition treatment on the residual variance (see results). We therefore refitted the models after pooling the two treatments to estimate variance components with the largest possible data set.

Paternal effects result from genetic variation among pollen donors and can be used to estimate the additive genetic variance in the various traits. In the design used here, the additive genetic variance equals four times the paternal variance (Conner and Hartl, 2004). In order to compare the evolutionary potential of the different traits, we estimated trait evolvabilities as the mean-square scaled additive genetic variance: $e = \sigma_A^2/\overline{x}^2$ where σ_A^2 is the additive genetic variance and $\overline{x}^2$ is the trait mean squared (Hansen et al., 2003). Evolvability measures the expected proportional response per generation to linear directional

selection of unit strength (Hansen et al., 2003, 2011). We used the posterior distribution of the variance component analyses on the full data set to obtain evolvabilities and their 95% credible intervals (the 2.5% and 97.5% quantiles).

Results

Blossom seed set

Variation in blossom seed set was limited and, although blossoms pollinated under weak pollen competition tended to produce fewer seeds (average ±SE: 8.22 ±0.11 seeds) than those pollinated under strong pollen competition (8.49 ±0.12 seeds), this difference was not statistically significant (contrast between treatments ±SE = -0.27 ±0.15; see appendix 1 for model selection). In both treatments, we observed aborted seeds (empty seed coats), but the frequency of these aborted seeds did not differ between treatments: 17/ 800 and 22/815 in the low and high pollen-competition treatments, respectively. Furthermore, we found no evidence for specific male plants to be responsible for higher levels of abortion (Appendix 2). Because these aborted seeds were very light (<25mg) and strongly affected the within-blossom variance in seed mass, we removed them from the data set before further analyses.

Maturation time

We found no statistically significant effect of pollen competition intensity on seed maturation time (average maturation times were 39.75 ±0.35 and 40.34 ±0.36 days in the weak and strong pollen-competition treatments, respectively; contrast between treatments ±SE = -0.58 ±0.32). However, seeds produced by large blossoms matured faster than those produced by smaller blossoms as indicated by the negative effect of peduncle diameter on maturation time (β = -4.34 ±1.41 day mm^{-1}, Appendix 1).

The majority of the variation in maturation time occurred among blossoms within plants (Table 1), but the identity of the maternal plant still explained 25% of the variation in maturation time (Table 1). Paternal effects on seed maturation time were limited and not statistically significant (Appendix 2), the identity of the father explaining only 1.23% of the variance in maturation time (Table 1). This paternal effect represented an evolvability of the maturation time of 0.008%. Finally, the residual variance in maturation time tended to be lower under weak pollen competition, but this difference was not supported statistically, as indicated by the overlapping credible intervals of the estimates (Table 1).

Individual seed mass

Seeds produced under weak pollen competition were on average 3.2% heavier than those produced under strong pollen competition (Table 2). In both treatments, peduncle diameter, maturation time, and blossom seed set positively affected seed mass (Table 2), this latter effect being slightly more pronounced under strong pollen competition (Table 2, Appendix 1). Overall, variation in peduncle diameter explained 6.2% of the variation in seed mass, while the difference in maturation time and blossom seed set explained 4.6% and 3.1% of the variation in seed mass, respectively.

Most of the variation in seed mass occurred within plants, at the among-blossom level (Table 1). Maternal effects explained ca. 14% of the variation in seed mass and tended to be smaller under weak (5.9% of the variation) than under strong pollen competition (22.4% of the variation), although this difference was not statistically significant (Table 1). Similarly, paternal effects tended to be lower under weak than under strong pollen competition (1.6% vs 4.0%), but these effects were limited and not statistically significant (Appendix 2). This paternal effect corresponded to an evolvability in seed mass of 0.05%. Here also, the residual variation in seed mass was not affected by the pollination treatment (Table 1).

247

248 *Germination success*

249 Out of the 1410 seeds sown, 35 (2.5%) failed to germinate, and we found no evidence that
250 seeds produced under strong pollen competition germinated better than those produced under
251 weak competition (Appendix 1). We found also no evidence for either maternal or paternal
252 effects on germination success, but seeds that failed to germinate tended to originate from
253 specific blossoms and to be clustered in specific trays (Appendix 2).

254

255 *Seedling vigor at one month*

256 The pollination treatment had no consistent effect on seedling vigor at one month. While
257 seedlings from the weak pollen-competition treatment tended to have slightly bigger
258 cotyledons (mean ±SE cotyledon diameter; strong pollen-competition treatment: 32.57 ±0.37
259 mm; weak pollen-competition treatment 32.83 ±0.37 mm, contrast between treatments ±SE =
260 0.26 ±0.19), they tended to have slightly smaller leaves (strong pollen-competition treatment:
261 73.40 ±1.78 mm; weak pollen-competition treatment 72.74 ±1.78 mm; contrast between
262 treatments ±SE = -0.66 ±0.90), but none of these differences was statistically significant.
263 Paternal effects on seedling vigor at one month remained very limited (Table 1) but
264 tended to be statistically significant for cotyledon diameter, where they explained nearly 5%
265 of the total variance in cotyledon diameter (data from the two treatments polled, Table 1;
266 Appendix 2). The evolvability of cotyledon diameter reached nearly 0.1%. In contrast, we
267 found no evidence for either maternal or paternal effect on the size of the largest leaf
268 (Appendix 2). Paternal effects never explained more than 2% of the variance in the size of the
269 largest leaf and the estimated evolvability was 0.05%. For both cotyledon diameter and the
270 size of the largest leaf, most of the variance occurred among offspring from the same father

within the same blossom (residual variance), but we found no difference in this component of the variance between the two pollination treatments (Table 1).

Finally, we noticed that the cotyledon diameter averaged at the level of the blossom was positively correlated with the average seed mass ($r = 0.46$, $P < 0.001$, $df = 167$; Fig. 2), while the correlation between the average size of the largest leaf and average seed mass was weaker and statistically not significant ($r = 0.11$, $P = 0.16$, $df = 167$).

Discussion

Contrary to our expectations, pollen competition affected neither the mean nor the residual (within father) variance of seed characteristics and early sporophyte performances in *D. scandens*. These results contrast with previous observations on the same species where pollen competition positively affected the mass of seeds obtained by self-pollination (Armbruster and Rogers, 2004). A possible explanation is that the two treatments in the present study resulted in similarly strong pollen competition because of the large pollen load used. The pollen loads used in our study were similar to those used by Armbruster and Rogers (2004), and we believe that possible differences in the intensity of pollen competition between the two studies were not generated by the pollen loads used. However, our experiment differs from the experiment by Armbruster and Rogers (2004) in the average distance that pollen tubes had to travel before reaching the ovules. In Armbruster and Rogers (2004) this distance was the same in both treatments because the pollen from both treatments was deposited in the same average place on the side of the stigma. In our experiment, the pollen in the low-dispersion treatment was deposited on the stigma tip. Therefore the distance the pollen tubes had to travel was shorter than the average distance for the high-dispersion pollen that were deposited on the side of the stigma. This design may have reduced the difference in the intensities of competition between the two treatments. It is also possible that the high

296 concentration of pollen grains on the tip of the stigma had favored their germination
297 (Brewbaker and Kwack 1963) and therefore diminished differences in germination rate. If
298 pollen competition is also mediated by the germination rate, this effect may have reduced
299 difference among pollen in the strong pollen competition treatment and therefore limited the
300 difference in pollen-competition intensity between our two treatments.

301 Alternatively, the difference between the two studies could be the result of differences
302 between self and outcross pollen in their interaction with the pistil during pollen-tube growth.
303 It is well known that the pistil has an influence on the outcome of pollen competition via
304 pollen-pistil interactions (Cruzan, 1990, 1993; Marshall, 1998; and see Skogsmyr and
305 Lankinen, 2002 for review). If these interactions depend on the simultaneous presence of
306 recessive deleterious alleles in the pistil and in the pollen grain, we can expect a stronger
307 selection among self-pollen than among pollen from an unrelated donor, because the formers
308 will carry a higher proportion of recessive deleterious alleles that are the same as those
309 carried by the maternal parent. This hypothesis is in agreement with the expectation that, in
310 species with low or intermediate selfing rates, selection at the pollen stage reduces inbreeding
311 depression (Charlesworth and Charlesworth, 1992, and see Armbruster and Rogers, 2004;
312 Lankinen and Armbruster, 2007 for empirical support). Under this hypothesis, the
313 improvement of progeny quality via pollen competition will be greater when competition
314 occurs among self-pollen or pollen from individuals related to the maternal plant, than when
315 it occurs among pollen grains from a donor unrelated to the maternal plant. In our
316 experiment, we crossed individuals with low degrees of relatedness. Therefore, benefits of
317 pollen competition in terms of selection against genetic load (recessive deleterious alleles)
318 might have been limited. This hypothesis is further supported by the fact that the residual
319 variance in the different traits was not reduced under strong pollen competition, as expected
320 if the variation among pollen grains from a single donor was limited.

Although some studies reported an increase in early sporophytic performance with increasing pollen competition (e.g. Mulcahy and Mulcahy, 1975; Winsor et al., 1987; Bertin, 1990; Richardson and Stephenson, 1992; Palmer & Zimmerman, 1994; Quesada et al., 1996) others found no positive effect of pollen competition on offspring vigor (Snow, 1990; Lassere et al., 1996; Niesenbaum, 1999). Additionally, many of the early studies have been criticized for confounding effects of variation in pollen competition with the differential genetic-sampling effects of variation in pollen load (Charlesworth, 1988; Delph and Havens, 1998). It is noticeable that among all the studies cited above, only three (Mulcahy and Mulcahy, 1975; Lassere et al., 1996; Armbruster and Rogers, 2004) managed to vary the level of pollen competition at constant pollen load, as done in the current study. Furthermore, studies including a limited number of pollen donors or recipients of unknown relatedness possibly confounded the effect of pollen competition with the effect of purging suggested by theoretical models (Charlesworth & Charlesworth, 1992). Our study is therefore consistent with the general lack of evidence that demonstrates a marked effect of competition among pollen grains from a single donor on the progeny performance.

It is expected that consequences of pollen competition are more pronounced when competition occurs among several pollen donors than among pollen grains from a single donor (Marshall and Ellstrand, 1985). This prediction, however, depends on the presence of among-sire additive and non-additive genetic variation affecting the offspring phenotype. In our study, the weak paternal effects observed both on seed mass and seedling performances suggest that benefits of competition among pollen from multiple donors due to additive effects may be limited. This has been partly confirmed by the absence of difference in seed mass between crosses with single *vs.* multiple pollen donors (Pelabon et al. submitted). To what extent non-additive effects of pollen competition among multiple donors may affect offspring performance remains unknown, however.

Paternal effects on the different traits studied were always weak; the sire variance never explaining more than 5% of the total variance. Evolvabilities estimated from these effects ranged from 0.008% in maturation time to ca.0.1% in cotyledon diameter. These estimates are low compared to the median evolvability of 0.26% reported for a large number of quantitative traits (Hansen et al., 2011). This is even more pronounced for seed mass, where the evolvability of 0.05% is very low compared to the mean evolvability of 0.95% ±0.15% for life history traits (Hansen et al., 2011). These results are comparable to those obtained in studies analyzing paternal effects in natural populations (Marshall and Ellstrand, 1986; Marshall, 1988; Marshall and Whittaker, 1989; Fenster, 1991; Byers et al., 1997; Pasonen et al., 2001; Diggle et al., 2010), but contrast with the large paternal effects on seed mass reported in *Arabidopsis thaliana* (House et al., 2010; de Jong et al., 2011). However, paternal effects in *A. thaliana* were estimated from crosses among ecotypes (i.e. among individuals from different populations), and they may not be comparable to any within-population paternal effects (see Pélabon et al., submitted for further discussion).

Overall, competition among pollen grains from a single donor unrelated to the maternal plant seems to have little effect on the quality of the progeny. Additionally, evolvabilities of seed mass and early sporophytic performances appear limited as well. This suggests that, in *D. scandens*, but also in other species of angiosperms, pollen competition may essentially reveal non-additive genetic effects. This raises questions concerning the role pollen competition may have as a mechanism of sexual selection (Marshall, 1998). We notice, however, that our measures of quality, as is the case in most studies, only concern early sporophytic performances. Paternal effects on late performances may be important and should be investigated.

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Table 1 – Variance components for maturation time (days), seed mass (mg), cotyledon diameter (mm) and the size of the largest leaf (mm) for each pollen-competition treatment and for the two treatments pooled (full data set). We provide the mean value of the posterior distribution of the variances with their 95% credible intervals obtained from the MCMCglmm R package. For maturation time, the residual variance correspond to the variation among blossom within mother, while for seed mass, cotyledon diameter and size of the largest leaf, the residual variance correspond to the variation among seeds within blossom. Evolvabilities were estimated from the median value of the posterior distribution of the paternal variance.

Variable	Components	Weak pollen competition		Strong pollen competition		Full data set	
		Var (95% CI)	% var	Var (95% CI)	% var	Var (95% CI)	% var
Maturation time	Mother	1.80 (0.23; 3.82)	28.83	1.23 (6.41 $\times10^{-8}$; 3.38)	14.16	1.84 (0.43; 3,51)	25.26
	Father	0.32 (1.58 $\times10^{-7}$; 1.12)	4.98	0.27 (5.94 $\times10^{-8}$; 1.12)	3.05	0.088 (3.9; 0.37)	1.23
	Residual	3.90 (2.56; 5.04)	66.19	6.83 (4.24; 9.21)	82.79	5.13 (4.05; 6.19)	73.51
	Evolvability					0.0082 (9.81$\times10^{-7}$;0.094)	
	CV	6.05%		6.72%			
Seed mass	Mother	1.39 (0.00;4.03)	5.91	4.20 (0.26; 8.72)	22.37	2.55 (0.31; 5.18)	14.39
	Blossom/mother	11.80 (8.17; 15.77)	68.92	8.51 (6.66; 11.90)	49.75	10.78 (8.65; 13.46)	62.69
	Father	0.65 (0.00; 2.65)	1.59	1.09 (0.00; 3.34)	4.03	0.43 (1.01 $\times10^{-7}$;1.52)	2.43
	Residual	3.57 (3.22; 395)	20.84	3.437 (3.10; 3.84)	20.47	3.50 (3.24; 3.76)	20.48
	Evolvability	0.067 (0.000; 0.920)		0.167 (0.001; 1.234)		0.052 (2.47 $\times10^{-8}$;0.37)	
	CV	9.78%		10.39%			
Cotyledon diameter	Mother	0.45 (0.00; 1.00)	7.01	0.50 (0.00; 1.28)	5.76	0.49 (0.093; 1.022)	7.48
	Blossom/mother	0.66 (0.19; 1.12)	11.64	1.49 (0.65; 2.43)	19.45	0.91 (0.53; 1.28)	14.02
	Father	0.19 (0.00; 0.63)	2.18	0.30 (0.00; 1.06)	2.47	0.33 (1.05$\times10^{-3}$; 0.95)	4.87

	Residual	4.31 (3.80; 4.79)	77.77	5.17 (4.56; 5.80)	69.92	4.77 (4.37; 5.15)	73.62
	Evolvability					0.095 (4.24×10^{-4}; 0.38)	
	CV	11.63		10.97			
Size largest leaf	Mother	7.44 (0.00; 18.16)	4.80	4.33 (0.00; 13.90)	2.41	2.54 (2.6×10^{-6}; 7.33)	2.02
	Blossom/mother	15.07 (5.52; 27.60)	11.01	23.49 (9.95; 38.16)	18.65	20.69 (12.24; 28.70)	16.65
	Father	2.31 (0.00; 9.13)	0.74	4.50 (0.00; 16.29)	2.03	1.68 (2.1×10^{-8}; 5.90)	1.32
	Residual	106.30 (94.59; 119.30)	81.75	91.64 (81.88; 103.50)	74.60	99.10 (91.32; 107.90)	80.01
	Evolvability					0.055 (1.26×10^{-9}; 0.36)	
	CV	22.34		19.79			

Table 2 – Parameter estimates for the model testing the effects of the pollen-competition treatment, peduncle diameter, maturation time and blossom seed set on seed mass. Covariates are mean centered so that the intercept of the model represent the mean seed mass in each pollen competition treatment (this analysis is based on 1726 seeds produced by 211 blossoms). Parameter estimates were obtained using the highest ranked model (see Appendix 1) fitted with REML. We removed the main intercept and the main effect for blossom seed set in order to obtain parameter estimates within each treatment.

Parameter	Estimate ±SE
Intercept (weak pollen competition) mg	40.94 ±0.49
Intercept (strong pollen competition) mg	39.66±0.49
Peduncle diameter	5.65 ±2.10
Maturation time	0.29 ±0.11
Blossom seed set (weak pollen competition)	0.43 ±0.30
Blossom seed set (strong pollen competition)	1.34 ±0.38

Fig.1 Blossom of the Dalechampia scandens (a) Complete blossom in the female phase when all male flowers are closed and the female flowers receptive. (b) Strong pollen competition resulting from the low dispersion of the pollen on the tip of the stigma (c) weak pollen competition resulting from the high dispersion of the pollen along the lateral surface of the pistil.

FIGURE 1 Blossom of *Dalechampia scandens*. (A) Complete blossom in the female phase when all male flowers are closed and female flowers receptive. (B) Strong pollen competition resulting from the low dispersion of the pollen on the tip of the stigma. (C) weak pollen competition resulting from the high dispersion of the pollen along the lateral surface of the pistil.

Fig. 2 Correlation between the cotyledon diameter and the seed mass in *D. scandens*. Each point represents blossom averages for both variables.

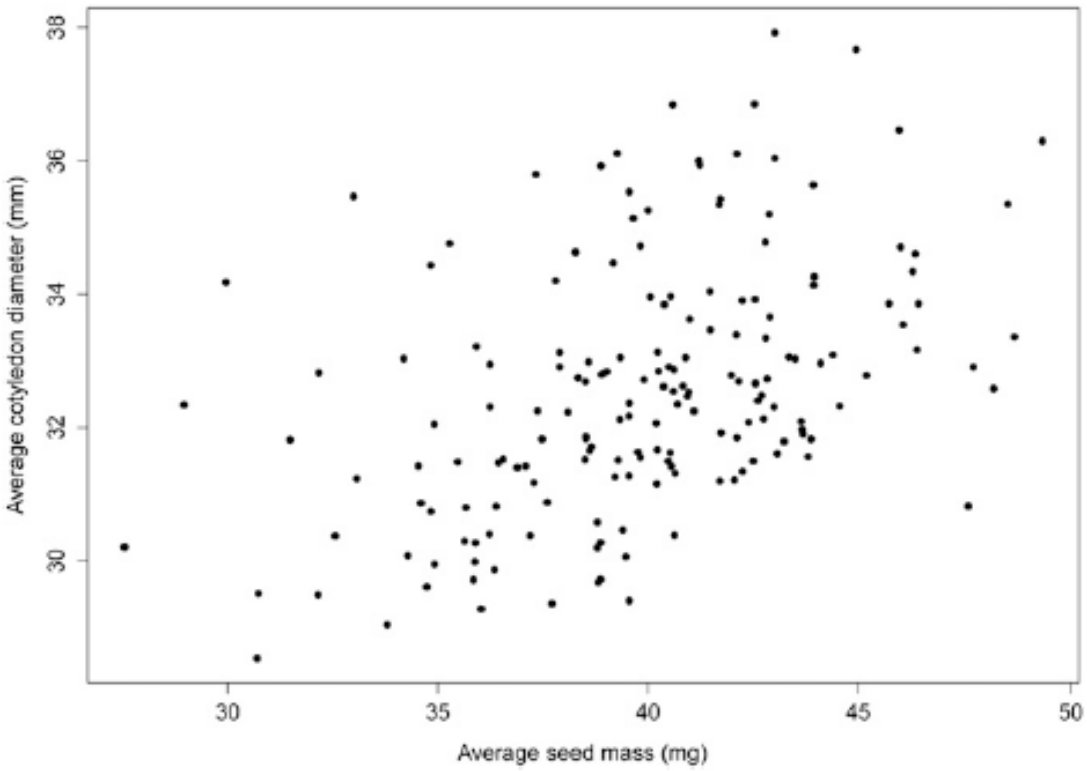

FIGURE 2 Correlation between the cotyledon diameter and the seed mass in *Dalechampia scandens*. Each point represents blossom averages for both variables.

Appendix 1 – Model selection for testing the effects of the fixed factors on blossom seed set, maturation time, seed mass, cotyledon diameter and the size of the largest leaf. AIC values are obtained from mixed-effects models fitted with ML. For blossom seed set and maturation time, random factors were male identity and female identity. For seed mass the random factors were male identity, female identity and blossom nested in female. K: number of parameters estimated. The sign × indicates an interaction between two explanatory variables. Models with the lowest AIC are written in bold. The predictor variables are treatment (Treat), peduncle diameter (Pdiam), maturation time (Mat) and blossom seed set (Seed set).

Models (predictor variables)	K	AIC	ΔAIC	AIC weight
Blossom seed set				
Treat	**5**	**585.62**	**0**	**0.63**
Constant	4	586.70	1.09	0.37
Maturation time				
Treat + Pdiam	**6**	**866.74**	**0**	**0.63**
Pdiam	5	867.79	1.06	0.37
Treat	5	897.29	30.56	0
Constant	4	898.01	31.28	0
Seed mass				
Pdiam.+ Seed set + Treat + Mat + Treat × Pdiam + Treat × Seed set + Treat × Mat	12	6961.18	3.53	0.06
Pdiam + Seed set + Treat + Mat + Treat × Pdiam + Treat × Mat	11	6962.77	5.12	0.03
Pdiam + Seed set + Treat + Mat + Treat × Pdiam + Treat × Seed set	11	6959.49	1.84	0.14
Pdiam + Seed set + Treat + Mat + Treat × Seed set + Treat × Mat	11	6959.44	1.79	0.14
Pdiam + Seed set + Treat + Mat. + Treat × Pdiam	10	6960.84	3.19	0.07
Pdiam + Seed set + Treat + Mat + Treat × Seed set	**10**	**6957.65**	**0**	**0.34**
Pdiam + Seed set + Treat + Mat + Treat × Mat	10	6961.27	3.62	0.06
Pdiam + Seed set + Treat + Mat	9	6959.27	1.62	0.15
Pdiam + Treat + Mat	8	6967.30	9.65	0.00
Pdiam + Seed set + Treat	8	6984.05	26.40	0.00

Seed set + Treat + Mat	8	7244.21	286.56	0.00
Pdiam + Seed set + Mat	8	6964.15	7.10	0.01
Germination success				
Treat	6	306.08	1.04	0.37
Constant	**5**	**305.04**	**0**	**0.63**
Cotyledon diameter				
Treat	7	6007.90	0.15	0.48
Constant	**6**	**6007.90**	**0**	**0.52**
Size of the largest leaf				
Treat	7	9998.20	1.48	0.32
Constant	**6**	**9996.71**	**0**	**0.68**

Appendix 2 – Model selection for testing paternal effects on the different variables. Models including or not paternal identity as a random factor are compared using AIC values obtained from models fitted with REML. Fixed effects are those from the highest ranked models in Appendix 1, and the random effect are presented in parentheses on the right side of the sign "|". The random effect (mother/ blossom) means blossom nested in mother.

Models	K	AIC	ΔAIC	AIC weight
Blossom seed set				
Constant | (father) + (mother)		589.8	1.8	0.29
Constant | (mother)		588.0	0	0.71
Maturation time				
Pdiam. | (father) + (mother)	5	865.73	2.11	0.74
Pdiam. | (mother)	4	863.62	0	0.26
Seed mass				
Pdiam.+ Seed set + treat + mat. + treat × Seed set | (father) + (mother /blossom)	10	6957.37	1.68	0.3
Pdiam.+ Seed set + treat + mat. + treat × Seed set | (mother /blossom)	9	6955.69	0	0.7
Germination success				
Constant | (tray) + (father) + (mother / blossom)	5	305.04	4.03	0.09
Constant | (tray) + (mother / blossom)	4	303.02	2.01	0.24
Constant | (tray) + (blossom)	3	301.01	0	0.66
Constant | (tray)	2	309.71	8.70	0.01
Constant | (blossom)	2	316.61	15.60	0.00
Cotyledon diameter				
Constant | (tray) + (father) + (mother / blossom)	6	6007.95	0	0.98
Constant | (tray) + (mother / blossom)	5	6015.77	7.83	0.02
Constant | (tray) + (blossom)	4	6026.93	18.99	0.00
Constant | (tray)	3	6136.58	128.63	0.00
Constant | (blossom)	3	6302.43	294.49	0.00
Size of the largest leaf				
Constant | (tray) + (father) + (mother / blossom)	6	9993.80	2.47	0.14

Constant | (tray) + (mother / blossom)	5	9991.87	0.53	0.37
Constant | (tray) + (blossom)	4	9991.33	0	0.49
Constant | (tray)	3	10072.17	80.84	0.00
Constant | (blossom)	3	10286.01	294	0.00

19